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PATENT Docket 488P1C3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
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Anderson *et al.*)
)
Serial. No. 08/036,014)
)
Filed: March 22, 1993)
)
For: DNA ENCODING VARIANTS OF TISSUE PLASMINOGEN ACTIVATORS AND EXPRESSION VECTORS AND HOSTS THEREFOR)

Group Art Unit: 1812

Examiner: M. Allen

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, DC 20231 on 23 November 1993
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AIDA A. MICHT
Name of Depositing Party

Aida A. Micht
Signature of Depositing Party

23 November 1993
Date of Signature

DECLARATION UNDER 37 C.F.R. §1.132

Honorable Commissioner of Patents and Trademarks
Washington, DC 20231

SIR:

I, Bruce A. Keyt, Ph.D., do hereby declare ~~and say as follows:~~

1. I hold a B.A. in Chemistry from Washington University and a Ph.D. in Biochemistry from Tufts University. I have particular specialization in thrombolytic protein biochemistry, having worked in the field for over 11 years at Genentech, Inc. My current title is Scientist, Department of Cardiovascular Research. I am a named inventor in the above-identified patent application. My scientific curriculum vitae is attached hereto as Exhibit A.

2. Table 1 attached hereto as Exhibit B summarizes the plasma clearance rates of t-PA variants disclosed in the above-identified application. The t-PA variants listed in the first column of Table 1 were generated and tested as described in this application, using the oligonucleotides shown on page 43, line 9, page 54, lines 2-12, and page 67, lines 10-22 of the specification. In the designation used, the amino acids are referred to using the one-letter code. Substituted amino acids are designated by identifying the amino acid occurring in wild-type human t-PA on the left side of a

particular amino acid position number, and identifying the substituted amino acid on the right side of the number. DNA encoding each variant was transfected in human embryonic kidney line 293C cells (Graham *et al.*, J. Gen. Virol., 36: 59 (1977)) for transient expression. All samples were tested as unpurified cell culture supernatants.

The clearance studies were done with radiolabeled samples of the t-PA variants using ^{125}I-YPACK. Radiolabeling was performed as described in this application. The results appearing in the second column of Table 1 are expressed as fractions of the plasma clearance rate of wild-type t-PA, which was assigned a value of 1.0.

3. As shown in Table 1, the plasma clearance rate of R250N t-PA was determined to be approximately 58% lower than the plasma clearance rate of wild-type t-PA.

4. Fig. 1 attached hereto as Exhibit C is a graphic depiction of the Table 1 data organized by location of the extra N-glycosylation site within the amino acid sequence of native human t-PA. As shown in Fig. 1, amino acid positions 101 to 107 in the t-PA molecule define a region in which the addition of extra N-linked glycosylation produces a plasma clearance rate that is at least 50% lower than the clearance rate of native human t-PA. Fig. 1 also shows that movement of the N-glycosylation site two or five amino acid positions downstream from the C-terminal end of the 101-107 amino acids region or two amino acid positions upstream from the N-terminal end of the region results in t-PA variants with clearance rates that are still significantly lower than that of native human t-PA. Hence, extra N-linked glycosylation in the region bounded by amino acids 99 to 112 yields t-PA variants with significantly reduced clearance.

5. Given the behavior of N-glycosylation sites in the 101-107 region, it is my considered scientific opinion that an N-

glycosylation site added anywhere within the region defined by amino acid positions 60 to 67 is reasonably expected to result in t-PA variants which when glycosylated, exhibit significantly slower plasma clearance than native human t-PA. Like the 101-107 region, the 60-67 region is less than 10 amino acids in length and is bounded by N-glycosylation positions which lower plasma clearance rate by approximately 50% or more. Thus, the 60-67 region is believed to define an activity center that extends for a few amino acids on either side of the region. Alternatively, amino acid positions 60 and 67 may each represent a separate activity center that extends for a few amino acids on either side. Both of these interpretations of the data presented in Table 1 and Fig. 1 indicate that a t-PA variant with an added N-glycosylation site and attached glycosylation in the region surrounding amino acid positions 60 and 67 will have an extended half-life. Therefore, a practitioner would reasonably conclude that a t-PA variant with N-linked glycosylation at a position anywhere between amino acid positions 57 to 61 or 63 to 69 as claimed in this application would have a significantly lower plasma clearance rate than native human t-PA.

6. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Nov. 23, 1993

Bruce A. Keyt

Bruce A. Keyt